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β-BUNGAROTOXIN INHIBITS A NON-INACTIVATING POTASSIUM CURRENT IN GUINEA PIG DORSAL ROOT GANGLION NEURONES

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β-Bungarotoxin (β-BuTx), at concentrations of 0.45–45 nmol/l, selectively reduced a portion of the non-inactivating potassium current (I_K) in dorsal root ganglion neurones of the guinea pig, measured by voltage clamp of internally perfused cells. The average reduction of I_K obtainable with β-BuTx was 34% and usually not completed within 20 min, but irreversible upon washing for 20 min. The I/V-characteristic of the current blocked by β-BuTx was almost linear. It is suggested that β-BuTx selectively blocks a non-inactivating subtype of potassium channel.

β-Bungarotoxin (β-BuTx) has been classified as a presynaptically acting toxin which impairs transmitter release at neuromuscular junctions due to its phospholipase-A activity [15, 16]. Electrophysiological data have revealed that the action of β-BuTx occurs in 3 stages [2, 8]: (I) an initial slight reduction of the amplitudes of evoked endplate potentials (EPPs), (II) an increase, and (III) a gradual decrease of EPP amplitudes until a complete block occurs. The last two steps are attributed to the phospholipase-A activity of the toxin. The facilitatory phase (phase II) is accompanied by a twitch augmentation in mouse diaphragm preparations [3].

Similar augmentation of muscle twitch has been described in the chick biventer cervicis muscle [5, 6] for dendrotoxin (DTx), a polypeptide isolated from the venom of the green mamba (*Dendroaspis angusticeps*), which has no phospholipase-A activity. Furthermore, DTx delays the neuromuscular block caused by β-BuTx [6]. From these findings and from structural homologies between DTx and the B-chain of β-BuTx it was suggested that both toxins may share a common binding site on motor nerve terminals [4, 6, 7], which has been recently confirmed by binding studies [12, 14]. We have previously demonstrated that DTx selectively inhibits a portion of the non-inactivating potassium current in sensory neurones of the dorsal root ganglion of guinea pigs [13]. Thus it seemed reasonable to examine whether β-BuTx and DTx had similar effects on ionic channels of sensory neurones. Our present results demonstrate that the effect of β-BuTx on outward potassium currents, although less pro-

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nounced, is strikingly similar to the action of DTx.

Experiments were performed on 23 isolated neurones from dorsal root ganglia (DRG) of guinea pigs (1–8 days old). The DRG were pretreated at 37°C for 20–50 min with trypsin (0.5%) and collagenase (Sigma, type III, 0.1%) in a solution containing (mmol/l): NaCl 138, KCl 2.6, HEPES 10; pH 7.4. After 45 min of washing with Earle's medium the DRG were mechanically isolated to obtain single cells.

The experimental set-up for voltage clamping and intracellular dialysis was similar to that described in detail by Kostyuk et al. [10]. Single cells were internally perfused with a solution, containing (mmol/l): KF 140, HEPES 10, buffered at pH 7.3. The external solution was composed of (mmol/l): NaCl 136, KCl 2.6, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 10; pH 7.4. Ca-currents and Ca-activated potassium currents were abolished by the fluoride ions [9]. Only neurones with stable membrane potentials between −45 and −65 mV whose inward sodium currents could be abolished by tetrodotoxin (TTx, 2 μmol/l) were used. However, we noted that neurones with TTx-resistant sodium currents [10] responded in a similar way to β-BuTx. All experiments were performed at room temperature (20–23°C). β-BuTx (T 2762, Sigma) was examined for homogeneity and found to be free of contaminants when assayed using disc-gel-electrophoresis.

Under our experimental conditions isolated neurones from guinea pig DRG displayed two distinct components of potassium outward currents [11] when subjected to depolarizing voltage shifts from a holding potential of −90 mV (Fig. 1A). The fast current component (which we will refer to as I_K^f) and the slow component (I_K^s) can be separated by shifting the holding potential to −50 mV. At this potential I_K^f

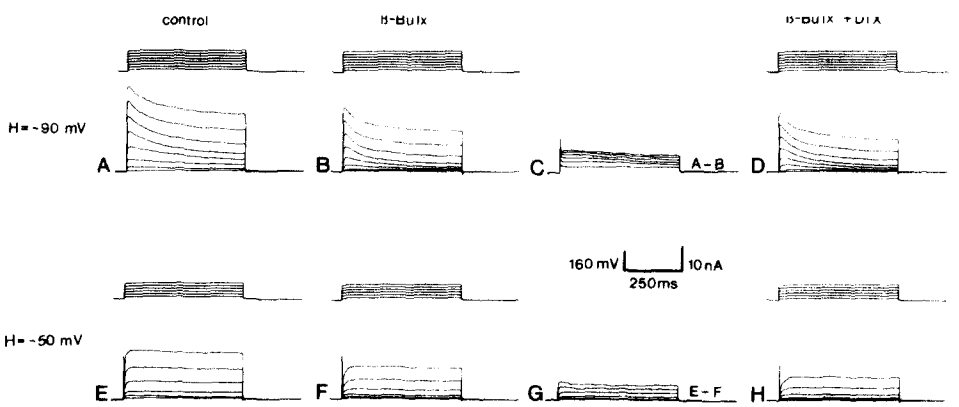

Fig. 1. Effects of β-BuTx and DTx on outward potassium currents. A and E: outward currents (lower traces) elicited by depolarizing voltage steps of 500 ms (upper traces) from a holding potential of H = −90 mV and H = −50 mV, respectively. B and F: outward currents 10 min after application of β-BuTx (45 nmol/l). C and G: currents blocked by β-BuTx at H = −90 mV and H = −50 mV, respectively. Leakage currents (less than 0.5 nA for a hyperpolarizing shift of 40 mV were compensated by an analogic circuit. Traces were obtained by digital subtraction of corresponding current traces (A and B) and (E and F) at depolarizing voltage shifts to −30/−10/+10/+30/+50/+70 mV. D and H: outward currents 10 min after the additional application of DTx (14 nmol/l).

is almost completely inactivated; thus voltage steps from −50 mV yield the non-inactivating, delayed outward current I_K^s (Fig. 1E).

The action of externally applied β-BuTx (45 nmol/l) on the two outward currents is demonstrated in Fig. 1B and F at a holding potential of −90 and −50 mV, respectively. The amount of current blocked by β-BuTx is almost identical at the two holding potentials (Fig. 1C, G). It appears that only a portion of I_K^s is inhibited by the toxin. On rare occasions also a slight reduction of I_K^f (less than 10%) was observed. Since this reduction usually occurred when investigating the cells for long periods of time, this may reflect the rundown of I_K^f.

Reduction of outward currents was obtained using concentrations of β-BuTx as low as 0.45 nmol/l. Since the development of the effect of β-BuTx was rather slow it was not possible to determine the maximal effect of β-BuTx at low concentrations; at higher concentrations (45 nmol/l) the action of the toxin usually had not ceased completely within 20 min. A typical example for the effect of different β-BuTx concentrations is shown in the I/V curves of Fig. 2A. Once the action of β-BuTx was completed no recovery of outward currents could be obtained by washing for up to 20 min.

The reduction of the non-inactivating potassium current obtained with β-BuTx (4.5–45 nmol/l, 10 min after application) was 34 ± 17% (mean ± S.D.; n = 9) for a depolarizing voltage shift to +50 mV from a holding potential of −50 mV. This is somewhat less than the maximal reduction of 47% obtainable with DTx (14 nmol/l) [13]. The additional application of DTx (14 nmol/l) further reduced the slow outward current as illustrated in Fig. 1D, H and the I/V curves in Fig. 2A. This effect may be due to an incomplete blockade of the relevant portion of the current by β-BuTx due to the slow time-course of its action.

To determine whether β-BuTx is as effective as DTx in suppressing the slow K-conductance, neurones were incubated for 1 h with β-BuTx (45 nmol/l). After this period the remaining outward currents could still be reduced by DTx by about 10–30% (n = 9) suggesting that β-BuTx is somewhat less effective than DTx in blocking I_K^s, even when sufficient time is allowed for β-BuTx to develop its maximal effect.

The I/V relationships of the β-BuTx-sensitive and β-BuTx-insensitive components of the slow potassium current are illustrated in Fig. 2B. It is evident that the I/V-characteristic of the current blocked by β-BuTx is quite different from that of the current insensitive to β-BuTx and from the current under control conditions (e.g. Fig. 2A). It should be noted that β-BuTx had no significant effects on currents elicited by hyperpolarizing pulses. The different I/V characteristics are similar to the results obtained using DTx [13] suggesting that the slow non-inactivating potassium is carried by two distinct subtypes of potassium channels: (I) a channel which is resistant to either of the toxins showing a non-linear I/V curve; (II) a channel which is sensitive to DTx as well as to β-BuTx (although to a lesser extent) and displays a rather ohmic behaviour. Another possible explanation for the current reduction, which cannot be ruled out by our experiments, may be an effect of the two toxins on the voltage sensitivity of potassium channels carrying I_K^s.

In conclusion β-BuTx can no longer be regarded as a toxin exclusively acting on

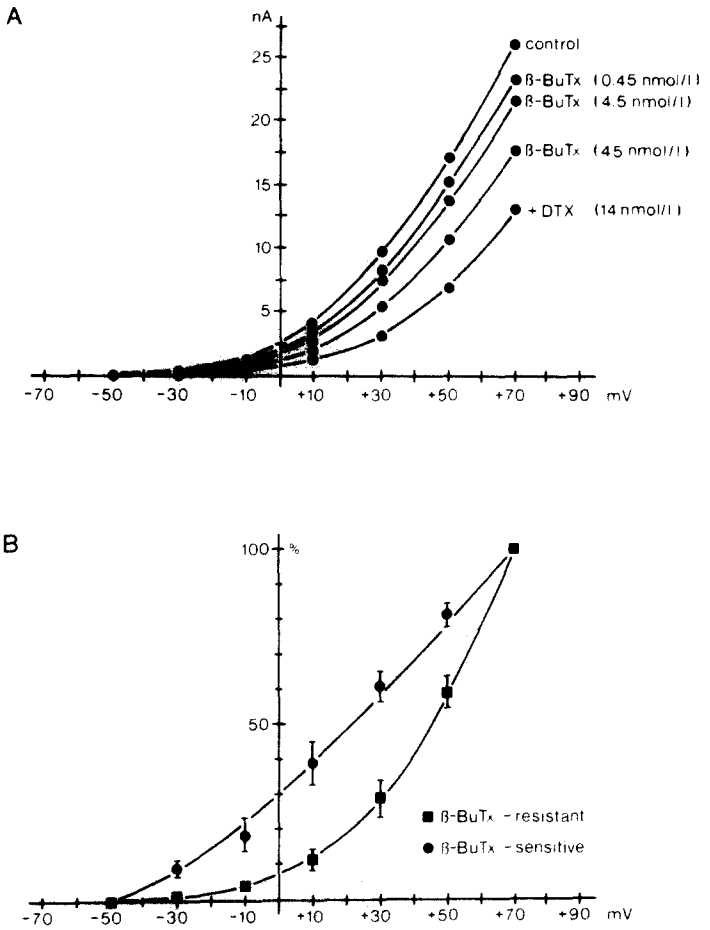

Fig. 2. Effect of β-BuTx and DTx on the I/V relationship of the non-inactivating potassium current I_K. Current amplitudes were measured at the end of depolarizing voltage steps lasting 500 ms from a holding voltage of −50 mV. A: I/V relationship of outward potassium currents before and 10 min after applicaton of β-BuTx and DTx as labelled in the graph. Data were acquired from the same cell as in Fig. 1. B: I/V relationships of currents blocked by β-BuTx (45 nmol/l, labelled β-BuTx-sensitive) and currents which remained after application of the toxin (labelled β-BuTx-resistant). For better comparison the currents were normalized by setting the current amplitude for a +70 mV shift to 100%. Values are means of 4 different cells (±S.E.M.). Curves were fitted by eye.

the transmitter releasing process itself. It may be inferred that a blockade of K-channels contributes to the transient facilitatory action of β-BuTx at neuromuscular junctions. Preliminary results indicate that presynaptic K-currents of mouse motor nerve endings are affected by β-BuTx (Penner and Dreyer, unpublished observations). Further experiments are required to determine whether the action of β-BuTx on the outward current is dependent on its phopholipase-A activity.

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